

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner: Kishore, G.

Applicant: De Meere et al

Serial No. 07/672,509

Filed: March 20, 1990

For: STABILIZED GONADOTROPIN  
CONTAINING PREPARATIONS

Group Art Unit 152

## DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of  
Patents and Trademarks  
Washington, D.C. 20231

Sir:

I, Dr. Arnold Titus Philip Skrabanja, declare the following:

1. I am a research scientist at Organon International, bv of Oss, The Netherlands.

2. I was awarded a doctorandus degree in biology by the University of Nijmegen (Nijmegen, The Netherlands) in 1980. I was awarded a Ph.D. in biochemistry by the same university in 1987. I performed two years of post-doctoral work at the Agricultural University of Wageningen (The Netherlands) in 1987 to 1989. I have worked since October 1, 1989 in the field of pharmaceuticals for Organon International, bv.

3. As part of my duties at Organon International, bv, I was requested to have certain work performed concerning the effect of surfactants on the stability and lyophilization of gonadotropins.

4. Pursuant to this request, I had certain gonadotropin containing solutions and lyophilisates made. These solutions and lyophilisates contained at least the gonadotropin hCG and the dicarboxylic salt sodium

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citrate. Certain lyophilisates also contained the non-ionic surfactant polysorbate 20 ("Tween 20"), the non-reducing sugar sucrose, the surfactant Macrogol 3400, or mixtures of some of these components.

5. The attached Figure 1 depicts the relative activity, as determined by an appropriate enzyme immunoassay ("EIA"), of recombinant source hCG solutions after contact with various described filters and tubes as would normally occur in everyday usage of all hCG solutions. This data shows that the addition of a non-ionic surfactant such as polysorbate 20 ("Tween 20") to the composition acts to preserve the activity of the hCG and to prevent its adsorption onto a container's (e.g. a silicone tube's) walls.

6. Various hCG solutions were also lyophilized in accordance with the procedures described in the above reference patent application, and the resulting lyophilisates were either reconstituted immediately or were reconstituted after storage under various conditions.

7. The compositions of the various lyophilisates made are described in the attached Figure 2, as are their respective storage conditions, and activities after storage.

8. Figure 2 shows the effect of surfactants on hCG activity on the reconstituted solutions (after freeze-drying), and solutions reconstituted after storage for 4 weeks at  $-18^{\circ}\text{C}$  and  $50^{\circ}\text{C}$ . Some compositions contained urinary hCG (i.e. Formulations A-F) while others contained hCG of recombinant source (i.e. Formulations G-J). Figure 2 shows that compositions containing recombinant hCG (i.e. highly pure hCG) are less stable than compositions containing urinary source hCG. Figure 1 also shows that the compositions without surfactant

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(i.e. Formulations A and G) contain less active hCG than those which contain a surfactant after accelerated decomposition testing (i.e. after storage for 4 weeks at 50°C (122°F)).

9. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and believe are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application and any patent issuing thereon.

Date: 15/1/92



Dr. A. Skrabanja  
Oss, The Netherlands

Attachments: Figure 1  
Figure 2

FIGURE 1

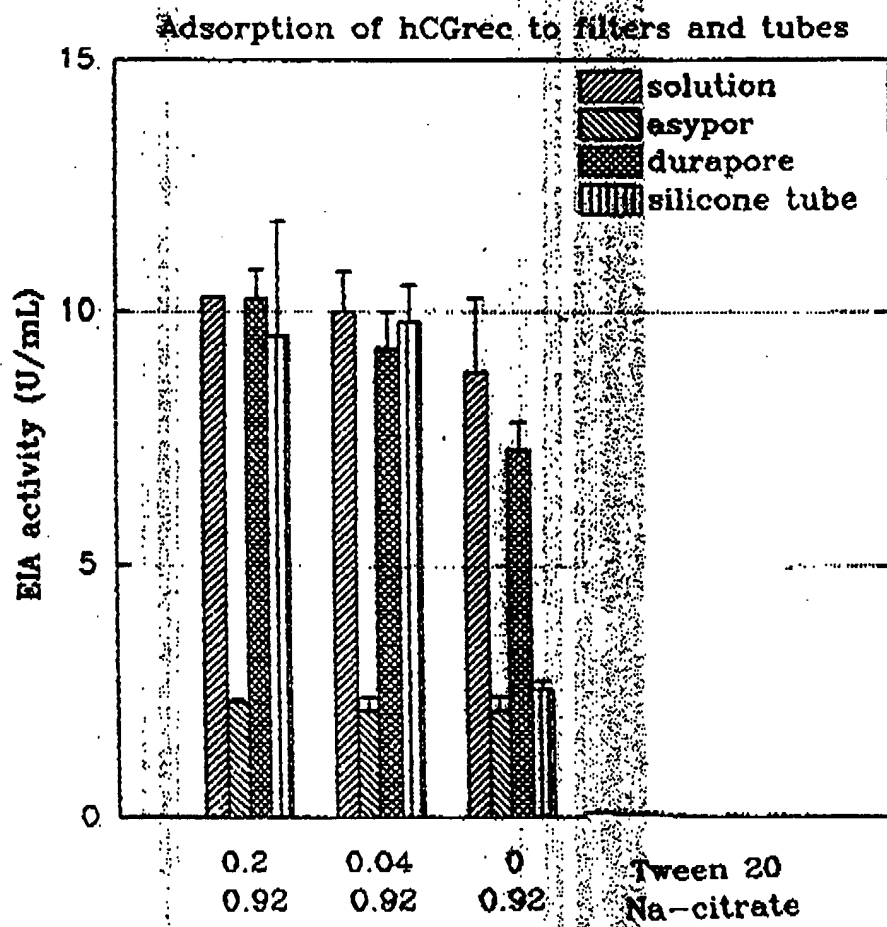
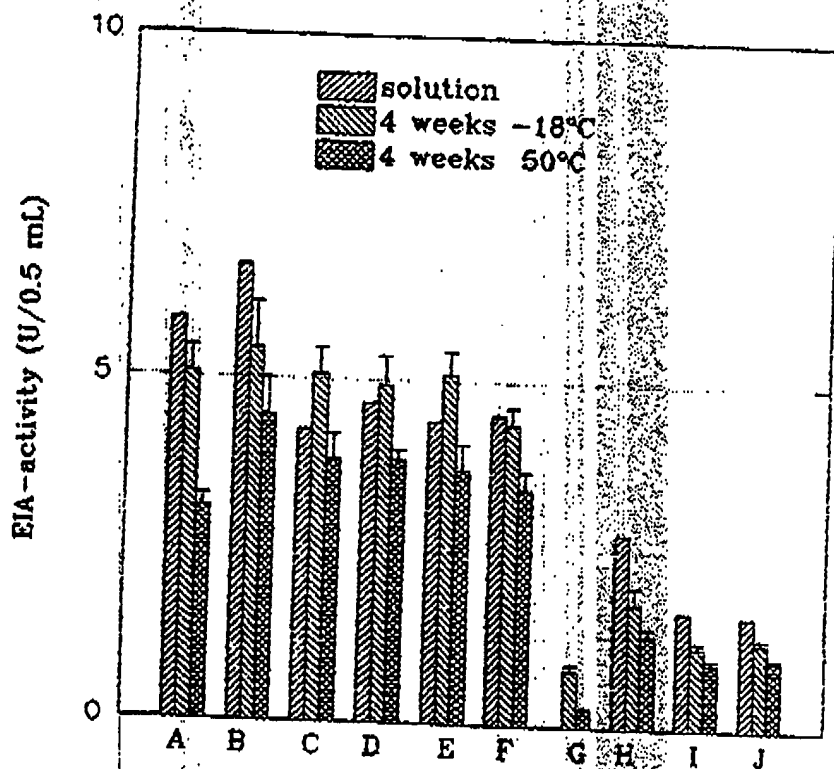


FIGURE 2



All formulations : 10 U/mL hCG (A-F uhCG; G-J hCGrec)  
 14.7 mg/mL Sodium citrate  
 50 mg/mL Sucrose

B 0.2 mg/mL Polysorbate 20  
 C 0.2 mg/mL Macrogol 3400  
 D 0.4 "  
 E 0.8 "  
 F 1.6 "  
 H 0.2 mg/mL Polysorbate 20  
 I 0.2 mg/mL Macrogol 3400  
 J 0.4 "